REMARKS

The objection to the drawings is noted. Since the objection relates only to the margins and legibility, no proposed drawing changes are believed to be necessary. Formal drawings will be submitted once the application has been allowed.

Claim 1 has been amended to delete indicate that the prokaryotic cells used in the method are "recombinant" prokaryotic cells. It is believed that this amendment does not constitute new matter, and its entry is requested.

In response to Applicants' arguments concerning the unity of invention of claims 1-20 of the present application in accordance with PCT Rule 13.1, the Examiner noted that the scope of claim 1 must be read to be broader in scope than claims 11 and 14 and new claims 21 and 22. Claim 1 has been amended so that the method uses "recombinant" prokaryotic cells. The "recombinant prokaryotic cells" contain "a DNA vector including a nucleotide sequence encoding a light producing enzyme under transcriptional control of a tetracycline repressor and a tetracycline promoter. In light of the amendment to claim 1, Applicants believe that it is now clear that all of the claims of the present invention, including claims 11, 14, 21 and 22 are directed to a single inventive concept under PCT Rule 13.1. Reconsideration of all of the claims is therefore requested.

The Examiner has rejected claims 1-10 and 16-19 under 35 USC §112, first paragraph for lack of enablement with respect to the scope of the claims. It is submitted that the Examiner is in error with respect to this rejection, especially with his analysis of the *Wands* factors.

The present claims are directed to a method for determining tetracycline in a sample, to recombinant prokaryotic cells and to a vector. The method utilizes the recombinant prokaryotic cells which contain the vector. The vector comprises a nucleotide sequence encoding a light producing enzyme under transcriptional control of a tetracycline repressor and a tetracycline promoter. Any light producing enzyme can be used, so long as it is under control of the tetracycline repressor and the tetracycline promoter. The nucleotide sequence can be placed in any vector which may be used in prokaryotic cells -- any prokaryotic cell which is compatible with the vector. To determine enablement, the specification is considered in light of the knowledge in the art at the time of the invention. One method that can be used, which is not the only method, is to consider the *Wands*

factors. If the *Wands* factors are properly considered in light of the knowledge in the art, it is submitted that the specification fully enables the claimed invention.

The Quantity of Experimentation Necessary

The Examiner states simply that several man-years of experimentation with little reasonable expectation of success would be needed, without providing any reasons or evidence to support his contention. Bald contentions without scientific reasons or evidence are not sufficient to sustain an enablement rejection. *In re Marocchi*, 169 USPQ 367 (CCPA 1971). There is little experimentation that is required, and even if some may be necessary, it is not an undue amount -- which is the proper standard. Recombinant DNA techniques are well established in the art, as evidenced by Sambrook et al. (*Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1989) and Ausebel et al. (*Current Protocols in Molecular Biology*, J. Wiley and Sons, N.Y., 1992, and periodic updates) and numerous volumes of *Methods in Enzymology*, among numerous other texts, all well known to persons of ordinary skill in the art. In view of the vast knowledge in the art, the preparation of vectors which are suitable for transforming prokaryotic hosts does not require undue experimentation. That is, skilled artisans know which vectors can be used with any given host. Transformation techniques for most prokaryotic hosts are also well known in the art. Thus, the transformation of hosts with such vectors also does not require undue experimentation. DNA encoding light producing enzymes and DNA containing tetracycline repressor and tetracycline promoter are well known in the art. In fact, Applicants have not invented any such DNA. Thus, the use of such DNA in preparing the vectors does not require any undue experimentation.

The use of *tet* system in other vectors and other hosts is shown by the following references. Copies of these references are provided with the accompanying Rule 132 Declaration of Dr. Matti Karp.

(a) *Bacillus subtilis*: Geissendorfer, M. and Hillen, W. (1990), *Appl Microbiol Biotechnol* 33:657-663;

(b) *Schizocaccharomyces pombe*: Faryar, K. and Gatz, C. (1992), *Curr Genet* 21:345-349;

(c) *Saccharomyces cerevisiae*: Dingermann, T. et al. (1992), *EMBO J* 11:1487-1492; Gari, E. et al. (1997), *Yeast* 13:837-848;

(d) *Mus musculus* myoblasts: Hofmann, A. et al. (1996), *Proc Natl Acad Sci USA* 93:5185-5190.

(e) *Mus musculus* brain: Mayford, M et al. (1996), *Science* 274:1678-1683;

(f) eukaryotes: Baron, U. et al. (1997), *Nucl Acids Res* 25:2723-2729; Hermann, B. and Gossen, M. (1998; filing date 1995), U.S. Patent No. 5,814,618; and

(g) *Mus musculus* fibroblasts: Holwell, T.A. et al. (1997), *J Cell Science* 110:1947-1956.

In addition, Rossi F.M.V. and Blau, H.M. (1998), *Curr Opin Biotechnology* 9:451-456 confirms such knowledge in its citation of previous publications (references 1, 2, 4, 5 and 6) showing that the *tet* system was well known to work in other vectors and other hosts. All of these references clearly demonstrate that the *tet* system was well known to work in host cells other than *E. coli* using vectors other than pTetLux1 and pTetLuc1. Thus, the preparation of vectors containing the elements disclosed in the specification, the preparation of host cells containing such vectors and the expression of the construct in such host cells require no undue experimentation.

Furthermore, the Examiner has not provided any sound scientific reasons to support his contention that the specification is not enabled for other vectors and hosts. In addition, the Examiner has not provided any sound scientific evidence to support his assertion that the quantity of experimentation is great, on the order of several man years. In the absence of such sound scientific reasons, the rejection must fail.

In addition, no undue experimentation is required to analyze samples of milk, fish, meat, infant formula, eggs, honey, vegetables, serum, plasma or whole blood. Techniques for analyzing samples from these materials were well known in the art at the time of the present invention. Furthermore, determination of the tetracycline in these materials, *albeit* by different assay methods, was well known at the time of the present invention. The following represent references which describe testing various samples for tetracycline. Copies of these references are provided with the accompanying Rule 132 Declaration of Dr. Matti Karp.

(a) beef liver, beef kidney, beef muscle, pork liver, pork kidney, pork muscle: Ikai, Y et al., *J. Chromatogr.* (1987) 411:313-323;

(b) beef liver, pork liver, chicken liver, beef muscle, pork muscle, chicken muscle, milk, egg, yellow tail, eel: Oka, H et al., *J. Chromatogr.* (1985) 325: 265-274;

(c) swine urine, swine plasma, swine liver: Sharma, J.P. and Bevill, R.F., *J. Chromatogr.* (1978) 166: 213-220;

(d) sheep urine, sheep plasma, cattle urine, cattle plasma: Sharma, J.P. et al., *J. Chromatogr.* (1977) 134:441-450;

(e) salmon: Carrignan, G. et al., *J. AOAC Int'l*, (1993) 76: 325-328;

(f) milk: Carson, M.C., *J. AOAC Int'l.* (1993) 76:329-334;

(g) bovine muscle, porcine muscle: Walsh, J.R. et al., *J. Chromatogr.* (1992) 596: 211-216;

(h) cattle muscle, cattle liver, cattle kidney, cattle blood, swine muscle, swine liver, swine kidney, swine blood: Moats, W.A., *J. Chromatogr.* (1986) 358: 253-259;

(i) catfish: Moretti, V.M. et al. *Analyst* (1994) 119:2749-2751; and

(j) honey: Oka, H. et al., *J. Chromatogr.* (1987) 400: 253-61.

Thus, the isolation and treatment of samples from milk, fish, meat, infant formula, eggs, honey, vegetables, serum, plasma or whole blood requires no undue experimentation.

Furthermore, the Examiner has not provided any sound scientific reasons to support his contention that the specification is not enabled for any type of sample regardless of heterogeneity. In addition, the Examiner has not provided any sound scientific evidence to support his assertion that the quantity of experimentation is great, on the order of several man years. In the absence of such sound scientific reasons, the rejection must fail.

The Amount of Guidance Provided in the Specification

The present specification provides sufficient guidance to a person of ordinary skill in the art to practice the claimed invention. The specification clearly describes the components of the vector present in the prokaryotic cells which are used to practice the claimed method. That is, the vector contains a nucleotide sequence which encodes a light-producing enzyme under the transcriptional control of a tetracycline repressor and a tetracycline promoter. Any vector can be used which contains such a nucleotide sequence, and any nucleotide sequence which contains the component parts can be used. The specification further provides guidance that any prokaryotic cell can be used, such as disclosed at page 9, lines 7-17. The specification provides guidance that the method can be used to assay for tetracycline in samples of milk, fish, meat, infant formula, eggs, honey, vegetables,

serum, plasma or whole blood. Thus, the specification provides guidance to a skilled artisan for the breadth of the claimed subject matter.

All of the novel aspects of the invention have been disclosed by the specification. The novel aspect is the use of a prokaryotic cell containing a vector for the determination of tetracycline in a sample. The vector comprises a nucleotide sequence encoding a light producing enzyme under the transcriptional control of a tetracycline repressor and a tetracycline promoter. Each of these elements is known to a skilled artisan. The novel aspect of the gene for a light producing enzyme being under the transcriptional control of a tetracycline repressor and tetracycline promoter is fully disclosed in the specification. Thus, Applicants have supplied the novel aspects of the invention, and the specification does not rely on the knowledge of a skilled artisan to supply any novel aspects.

The Presence or Absence of Working Examples

The specification provides working examples which demonstrate that there is no undue experimentation required to determine the presence of tetracycline in accordance with the present invention. Thus, it should be noted that the application discloses the determination of tetracycline from two especially difficult sample matrixes, i.e. from pig serum (Example 3 and Fig. 9) and cow milk (Example 4 and Fig. 10). Tested pig serum was highly lipemic and milk is generally known to be a very difficult sample matrix for analytical purposes. A person skilled in the art (PSA) is, however, able to use the teachings of the application, i.e. the biosensor disclosed, for determining tetracycline from other samples by incorporating general knowledge of the pretreatment of each specific kind of sample, which knowledge would be available to the PSA (see above). Instruction and/or guidelines for pretreatment of samples can be found in textbooks and numerous articles. The application discloses the biosensor and a few real sample matrixes in which the presence of tetracycline has successfully been demonstrated.

The examples in the specification clearly demonstrate that the need for cleanup of samples by pretreatment is minimal. It should also be noted that the actual reaction which produces the light to be measured takes place within a bacterial cell. A bacterial cell is known to maintain the microenvironment within the cell to be very stable, e.g. its intracellular pH would remain highly stable also if the pH of its environment were to fluctuate significantly. Thus, the biosensor of the invention is highly applicable for different sample matrixes.

The examples and specification disclose a prototypic determination method, which has been shown to enable determination of tetracycline from a few real sample matrixes. It has also been disclosed how the biosensor has been obtained and how it is used. A PSA can use the biosensor for determination of tetracycline from different kinds of samples, taking into account known guidelines for determination of tetracycline from different kinds of samples (as referred to above). The application also provides guidance on how a PSA can easily make the assay more sensitive if needed.

Nature of the Invention

The present invention is in the area of recombinant DNA and assay techniques. Most aspects of recombinant DNA are no longer inherently unpredictable. For example, a skilled artisan can predict with reasonable certainty that a vector useful for a specific host cell can be used in a host cell regardless of the gene inserted into the vector. Thus, for the factors of the present invention, the degree of unpredictability is low. Furthermore, the specification clearly provides a high level of enablement through the working examples which demonstrate the practice of the claimed invention.

The State of the Prior Art

Techniques for preparing recombinant DNA vectors and transfected host cells were well known at the time of the present invention. The Examiner is certainly well aware of the conventional texts in this area, including the following: Maniatis *et al.*, 1982, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; Sambrook *et al.*, 1989, *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; Ausubel *et al.*, 1992, *Current Protocols in Molecular Biology*, J. Wiley and Sons, NY; Glover, 1985, *DNA Cloning*, **I** and **II**, Oxford Press; *Nucleic Acid Hybridization* (B. D. Hames & S. J. Higgins eds. 1984); *Transcription And Translation* (B. D. Hames & S. J. Higgins eds. 1984); B. Perbal, *A Practical Guide To Molecular Cloning* (1984); the treatise, *Methods In Enzymology* (Academic Press, Inc., N.Y.); and *Gene Transfer Vectors For Mammalian Cells*, J. H. Miller and M. P. Calos eds., 1987, Cold Spring Harbor Laboratory. These well known texts describe numerous vectors useful for transfecting numerous host cells, and the well known consideration that any given vector is used for a host in which the vector is capable of replication. These well known texts also describe numerous expression systems for the production of products of cloned nucleic acids.

As discussed in great detail above, the state of the art with respect to recombinant DNA is very broad. In this context, the state of the prior art with respect to vectors, host cells, DNA sequences for light producing enzymes, tetracycline repressor and tetracycline promoter is not limited in any respect. In addition, techniques for preparing such vectors and host cells containing the vectors are not limited in any respect. Thus, it is submitted that applications of these techniques to the claimed method are not limited in any respect.

The Relative Skill in the Art

The relative skill in the art is lower than that suggested by the Examiner. The skill in the art at the time that recombinant DNA inventions were first made, e.g. in the 1970's, was certainly at the level of individuals with a Ph.D. in biochemistry. However, such a level of skill for the ordinary artisan is no longer that high. Undergraduate students today routinely perform experiments in the recombinant DNA art. These students can readily isolate and clone sequences when provided with sufficient information as to what is to be isolated and cloned. Texts are available in the art which provide all of the necessary instructions for making recombinant DNA, recombinant cells and growing such recombinant cells. The Examiner is no doubt well aware of Sambrook et al., *supra*, Ausebel et al., *supra*, and the numerous volumes of *Methods in Enzymology*. These references were well known to persons of skill in the art at the time of the invention. Thus, a PSA would at least know the basics of microbiology and molecular biology, which would enable him to practice the claimed invention on the basis of the present disclosure.

The Breadth of Scope of the Claims

The Examiner contends that the claims are broad on the basis of several factors. Applicants agree that the claims encompass any number of constructs that can be used in the detection of tetracycline. This factor in and of itself does not render the claims enabled by the specification. Even assuming that the claims could be considered as broad as the Examiner considers, each of the factors enumerated by the Examiner is well known to a skilled artisan and described in the prior art. For example, the prior art fully understands each of the factors noted by the Examiner as follows.

Factors (a) and (b): Sambrook et al. referred to in the application (and cited above) gives detailed instructions on e.g. how to prepare, propagate and control genetically modified organisms (GMO).

Factor (c): For example, the publications referred to above describe the pretreatment of different kinds of samples and the influence of environmental parameters on tetracycline determination. Thus, a PSA would know what should be observed for each individual type of sample when determining tetracycline from different kinds of samples.

Factor (d): The assay is group-specific. It does not determine which specific tetracycline is involved.

Factor (e): The assay is a screening assay and is not intended to be used for quantitative assays. The biosensor can be used for screening. It gives a qualitative, i.e. a positive or negative, result. No quantitation has been disclosed in the application or claimed in the claims.

Factor (f): The level of the sensitivity of the assay is demonstrated by the examples.

Thus, as demonstrated above, the assay system is predictable, significant guidance has been provided by the specification, working examples demonstrating the invention have been provided, the state of the art is well developed, the level of skill in the art is not high, the claims are more limited breadth than asserted by the Examiner for several of the noted factors. Thus, a proper application of the *Wands* factors demonstrates that the specification is fully enabling for the claimed subject matter and that no undue experimentation is necessary to practice the claimed invention. For all of the above reasons, it is submitted that the specification is fully enabling to a person of ordinary skill in the art.

To further demonstrate that the present claims are fully enabled by the specification and to demonstrate the ordinary skill in the art, Applicants submit a Rule 132 Declaration of Dr. Matti Karp. Applicants believe that this Rule 132 Declaration clearly shows the state of the art at the time of the present invention, both with respect to (a) assaying any type of sample regardless of heterogeneity and (b) other vectors and host cells, was such so that the specification is fully enabling for the present claims. This Rule 132 Declaration discusses the references noted above, which are provided with the accompanying Information Disclosure Statement. It is believed that this sworn statement in a declaration fully rebuts the scientifically unsupported conclusions reached by the Examiner in his rejection.

In view of the above amendments and remarks, it is believed that the claims satisfy the requirements of the patent statutes and are patentable over the cited prior art. Reconsideration of the

instant application and early notice of allowance are requested. The Examiner is invited to telephone the undersigned if it is deemed to expedite allowance of the application.

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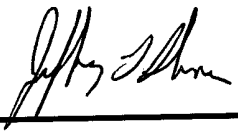
<tr><td>SIGNATURE</td><td colspan="2">  </td><td>DATE</td><td colspan="2">28 December 2001</td></tr>
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</table>

Attachments: Marked-Up Copies of Amendments

Marked-up Copy of Amended Claim

1 (twice amended). A method for the determination of tetracycline in a sample characterized in that

- the sample is brought into contact with recombinant prokaryotic cells encompassing a DNA vector including a nucleotide sequence encoding a light producing enzyme under transcriptional control of a tetracycline repressor and a tetracycline promoter,

- detecting the luminescence emitted from the intact cells, and

- comparing the emitted luminescence to the luminescence emitted from cells in a control containing no tetracycline

- wherein a detectable luminescence higher than a luminescence of the control indicates the presence of tetracycline in the sample.